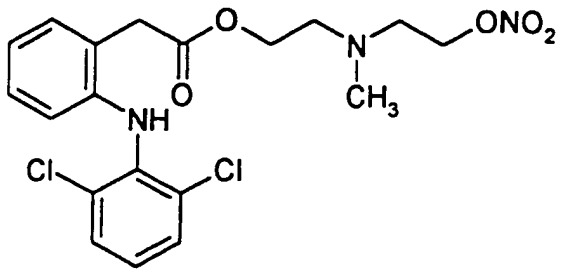
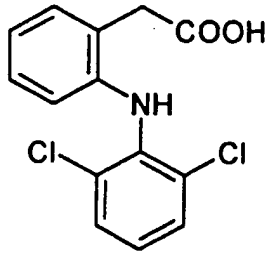
EXAMPLE 14

Preparation of 2-[(2,6-dichlorophenyl)amino]benzene acetic acid [N-methyl-N-(2-hydroxyethyl)]-2-aminoethyl ester (E-14)



The precursor drug is diclofenac of formula:



(E-14a)

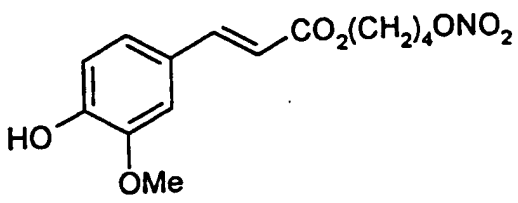
The precursor compound of B is N-methyldiethanolamine of formula $HO-(CH_2)_2-N(CH_3)-(CH_2)_2-OH$.

The compound is synthetized according to the procedure described in Example 5. Yield: 52%.

Elementary analysis:	C	H	N	Cl
Calculated	51.60%	4.78%	9.50%	16.03%
Found	51.60%	4.77%	9.53%	16.04%

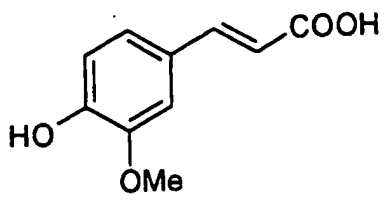
EXAMPLE 15

Preparation of 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid 4-(nitroxy)butylester



(E-15)

The precursor drug is ferulic acid of formula (E-15a)



(E-15a)

The precursor compound of B is 1,4-butandiol.

a) Preparation of 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid 4-bromo butyl ester

To a solution of ferulic acid (10 g, 51.51 mmoles) in tetrahydrofuran (400 ml), triphenylphosphine (27 g, 103 mmoles) and carbon tetrabromide (34.1 g, 103 mmoles) are added. The reaction mixture is maintained under stirring at room temperature for 4 hours, then filtered and evaporated under reduced pressure. The reaction crude product is purified by chromatography on silica gel, eluting with n-hexane/ethyl acetate 7/3 (ratio by volume). 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid 4-bromo butyl ester is obtained.

b) Preparation of 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid 4-(nitroxy)butyl ester

To a solution of 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid 4-bromobutyl ester (2.72 g, 6.89 mmoles) in acetonitrile (25 ml) silver nitrate (1.48 g, 8.71 mmoles) is added. The reaction mixture is maintained under stirring and heated at 80°C for 7 hours away from light, then cooled at room temperature, filtered to remove the silver salts and evaporated under reduced pressure. The residue is purified by chromatography on silica gel, eluting with n-hexane/ethyl acetate 7/3 (ratio by volume). 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid 4-(nitroxy) butyl ester
is obtained. Yield: 56%.

Elementary analysis:	C	H	N
Calculated	54.02%	5.50%	4.50%
Found	54.00%	5.52%	4.49%

PHARMACOLOGICAL TESTS

EXAMPLE

Acute Toxicity

Acute toxicity has been evaluated by administering to a group of 10 rats weighing 20 g a single dose of each of the compounds to be tested, by cannula, by os in an aqueous 2% w/v suspension of carboxymethylcellulose.

The animals are kept under observation for 14 days. In no animal of the group toxic symptoms appeared even after a 100 mg/Kg dose administration.

EXAMPLE F1

Test 1 - experimental model in vivo with N-ethylmaleimide (NEM): study of the gastric tolerability of some drugs screened as precursors of the compounds of the invention.

The animals (rats, weight about 200 g) are distributed in the following groups (No. 10 animals for group):

A) Control groups:

1° group: treatment: only carrier (aqueous suspension 1% w/v of carboxymethylcellulose, dose: 5 ml/Kg when the drug is administered by os, physiologic solution when by parenteral route),

2° group: treatment: carrier + NEM,

B) Groups administered with each drug:

group I: treatment: carrier + drug,

group II: treatment: carrier + drug + NEM.

The drugs assayed in this experiment are the following (Table I): indomethacin, ambroxol, mesalamine, sodic alendronate, tacrine, omeprazol, misoprostol.

Indomethacin, ambroxol and alendronate are administered by os, mesalamine by intracolonic (rectal) route and tacrine, omeprazol, misoprostol by subcutaneous route.

The maximum tolerated dose, determined by administering each substance by the above said routes to the animals not treated with NEM, is reported in Table I. With higher doses than those reported in the Table, enteropathy, diarrhoea, depression, tremor and sedation have appeared in the animals.

In this experimental model the animals are at first treated with NEM by subcutaneous injection at a dose of 25 mg/kg in physiologic solution. The drug is administered one

hour later, in suspension in the carrier. Animals are sacrificed after 24 hours and evaluation of the damage to the gastrointestinal mucosa is made by counting the number of rats, inside each group, with lesions to the stomach at a visual inspection. The total number of said rats is then divided by the total number of rats of the group and multiplied by 100. The thus obtained percentages are reported in Table I. The Table shows that in the groups of rats treated with said drugs without NEM, no gastric lesions were detectable.

All the rats of group II (treated with NEM) showed gastric lesions after administration with the following drugs: indomethacin, ambroxol, mesalamine, sodic alendronate, tacrine. Said drugs therefore can be used in the synthesis of the products of the invention.

Omeprazol and misoprostol cannot instead be used, on the basis of the results provided in test 1, for preparing the products of the invention.

EXAMPLE F2

Test 2 (in vitro): inhibition of apoptosis (DNA fragmentation) induced in the endothelial cells by CIP in the presence of some drugs screened as precursors of the compounds of the invention.

The following precursor drugs (Table II): indomethacin, paracetamol, clopidogrel, salbutamol, ambroxol, sodic alendronate, diphylline, cetirizine, enalapril, nicotinamide, ampicilline, aciclovir, mesalamine, tacrine, simvastine, omeprazol have been tested.

Human endothelial cells of the umbilical vein are prepared according to a standard method. Fresh umbilical veins are filled with a collagenase solution 0.1% by weight and incubated at 37°C for 5 minutes.

Subsequently the veins are perfused with the medium M 199 (GIBCO, Grand Island, NY) pH 7.4 with 0.1% (weight/volume) of collagenase, added with 10% of bovine fetus serum (10 mcg/ml), sodium heparin (50 mcg/ml), thimidine (2.4 mcg/ml), glutamine (230 mcg/ml), penicillin (100 UI/ml), streptomycin (100 mcg/ml) and streptomycin B (0.125 mcg/ml). The cells are collected from the perfusate by centrifugation at 800 rpm and harvested in culture flasks T-75, pretreated with human fibronectin. Cells are then harvested in the same medium,

added with bovine hypothalamic growth factor (100 ng/ml). When the cells of the primary cell culture (the cells directly removed from ex-vivo umbilical vein) form a single layer of confluent cells (about 8,000,000 cells/flask), harvesting is stopped and the layers are washed and trypsinized. The cellular suspensions are transferred into wells of a culture plate having 24 wells, half of said wells being added with the same culture medium containing the drug at a 10^{-4}M concentration, and harvested in a thermostat at 37°C at a constant moisture (90%), CO_2 = 5%. When the drug is not soluble in the culture medium, it is formerly dissolved in a small amount of dimethylsulphoxide. The maximum amount of dimethylsulphoxide which can be added to the culture medium is 0.5%. Only the cells coming from these first subcultures are used for the tests with cumene hydroperoxide (CIP). The cells are identified as endothelial cells by morphological examination and by the specific immunological reaction towards factor VIII; these cultures did never show contaminations from myocytes or fibroblasts.

Before starting the test, the cellular culture medium is removed and the cellular layers are carefully washed with a standard physiologic solution buffered with phosphate 0.1 M pH 7.0, at the temperature of 37°C. The content of each well is then incubated for one hour with a CIP suspension in the culture medium at a 5 mM concentration. Evaluation of the cellular damage (apoptosis) is carried out by determining the per cent variation of the DNA fragmentation in the cultures containing the drug + CIP with respect to the controls treated with CIP only. Said % variation of DNA fragmentation is determined by evaluating the fluorescence variation by a BX60 Olympus microscope (Olympus Co., Roma) set at the wave length of 405-450 nm, of the test samples with respect to the optical density of the controls. The fluorescence of each sample was determined on 5 replicates. Statistic evaluation has been made with t Student test ($p < 0.01$).

Results are given in Table II and show that indomethacin, paracetamol, clopidogrel, salbutamol, sodic alendronate, diphylline, cetirizine, enalapril, nicotinamide, ampicilline, aciclovir, tacrine, omeprazol do not significantly inhibit

apoptosis; these drugs can therefore be used for preparing the products of the invention.

On the contrary ambroxol, mesalamine and simvastatine inhibit apoptosis. Therefore on the basis of the results of test 2 these compounds could not be used for preparing the products of the invention.

EXAMPLE F3

Test 3 - experimental in vivo model with N^{w}-nitro-L-arginine-methyl ester (L-NAME): gastric tolerability (gastrointestinal damage incidence), hepatic (GPT dosage, glutamic-pyruvic transaminase) and cardiovascular (blood pressure) of some drugs screened as precursors of the compounds of the invention.

The experimental model adopted is according to J. Clin. Investigation 90, 278-281,1992.

The endothelial dysfunction is evaluated by determining the damage induced by L-NAME administration to the gastrointestinal mucosa, the hepatic damage (GPT increase), and the vascular endothelium or cardiovascular damage as blood hypertension.

The animals (rats, average weight 200 g) are divided in groups as herein below described. The group receiving L-NAME is treated for 4 weeks with said compound dissolved at the concentration of 400 mg/litre in drinking water. The following groups (No. 10 animals for group) are constituted:

A) Control groups:

1° group: treatment: only carrier (aqueous suspension 1% w/v of carboxymethylcellulose, dose: 5 ml/Kg when the drug is administered by os, physiologic solution when by parenteral route),

2° group: treatment: carrier + L-NAME,

B) Groups treated with the drug:

3° group: treatment: carrier + drug,

4° group: treatment: carrier + drug + L-NAME.

The drugs used in the test are paracetamol, doxorubicine, simvastatine, omeprazol and misoprostol. Each drug is administered once a day for 4 weeks.

The maximum tolerated dose of the drug being administered to the animals is determined by evaluating, in a separate dose scaling up experiment on untreated animals, the appearance in

the animals of symptoms such as enteropathy, diarrhoea, depression, tremor, sedation.

At the end of the four weeks access to water is prevented and after 24 hours the animals are sacrificed.

One hour before the sacrifice blood pressure is determined and a blood pressure increase is taken as an indication of a damage being occurred to vascular endothelium.

The damage to the gastric mucosa is evaluated as previously mentioned in test 1 (ex. F1). The hepatic damage is determined by evaluation after the sacrifice of the glutamic-pyruvic transaminase (GPT increase).

The drug meets test 3 and it can therefore be used for preparing the compounds of the invention, when in the group of rats treated with L-NAME + drug + carrier, an higher hepatic damage (higher GPT values) and/or higher gastric damage and/or higher cardiovascular damage (higher blood pessure) are found in comparison with the group treated with the carrier only, or the group treated with carrier + drug, or the group treated with carrier + L-NAME.

The test results are reported in Table IV. The % gastric lesions have been determined as in Test 1. The % GPT and % blood pressure values are referred to the corresponding value found in the animals of the 1st group of the control groups. The average value of the blood pressure in this group was of 105 $\pm$ 8 mmHg.

The results obtained show that paracetamol, doxorubicine and simvastatine cause hepatic damage and gastroenteropathy (GPT values and the gastric lesions are % higher compared both with the corresponding groups treated with the drug, in the absence of L-NAME, and with the controls treated with L-NAME).

These drugs can therefore be used for preparing the products of the invention.

Omeprazol and misoprostol should not instead be used, on the basis of this test, for preparing the products of the invention.

EXAMPLE F4

Test 4A: Activity of some substances used as precursors of B in the products according to the invention in inhibiting the haemolysis of erythrocytes induced by cumene peroxide.

Test 4a is performed according to the method described by R. Maffei Facino, M. Carini G. Aldini, M.T. Calloni, Drugs Exptl. Clin. Res. XXIII (5/8) 157-165 1997.

Erythrocytes isolated by using standard procedures from Wistar male rats (Charles River), are suspended in a physiological solution buffered at pH 7.4 with phosphate buffer and equilibrated at 4°C for 4 days. then an aliquot of said suspension is centrifuged at 1000 rpm for 5 minutes and 0.1 ml of the centrifuged erythrocytes are diluted to 50 ml with sodium phosphate buffer of the same above molarity, thus obtaining a suspension containing 0.2% by volume of erythrocytes. 3.5 ml portions of said diluted suspension are added of 0.1 ml of an alcoholic solution of cumene hydroperoxide 9.72 mM, which causes lysis of the cells. The resulting suspension is then incubated at 37°C. An increase of the turbidity is observed in the suspension. The process of cell lysis is followed by turbidimetry at 710 nm, by determining the optical density (or the transmittance) at intervals of 30 minutes. The time at which there is the maximum amount of cell lysed, that corresponds to the maximum turbidity of the suspension, is taken as the Tmax and it is assumed to correspond to a cell lysis of 100%. 0.2 ml of 38 mM ethanol solutions of the test compounds to be used as precursors of B are added to aliquots of 3.5 ml of the diluted suspension of erythrocytes above prepared, the resulting suspension preincubated for 30 minutes, 0.1 ml of an alcoholic solution of cumene hydroperoxide 10.26mM is then added, and at the time Tmax it is determined the percentage of haemolysis inhibition in the sample from the ratio, multiplied by 100, between the absorbance of the suspension of the sample containing the erythrocytes, the precursor of B and cumene hydroperoxide respectively and that of the suspension containing the erythrocytes and cumene hydroperoxide; the precursors of B meet the test if they inhibit the haemolysis induced by cumene hydroperoxide by a percentage > 15%;

In Table V are reported the results obtained with the following substances: N-methyldiethanolamine, diethylenglycol, thio-diethylenglycol, 1,4-butandiol, butanol and diethanolamine.

which it is shown that the compounds under test are ineffective in inhibiting the radical production from the iron ion.

Therefore these compounds can be used as precursor compounds of B for obtaining the compounds of the present invention.

EXAMPLE F6

It has been evaluated the activity of some of the compounds object of the present invention and of the corresponding precursor drugs in inhibiting DNA degradation (apoptosis) in endothelial cells exposed to the action of hydrogen peroxide (HP).

Hydrogen peroxide is a mild oxidant and is considered as an essential mediating agent in pathologies associated with oxidative stress (B. Halliwell, J. Gutteridge "Free Radicals in Biology and Medicine", page 416, 1993). Therefore the pharmacological activity of compounds to be used under oxidative stress conditions is evaluated through their capability of neutralizing the cytolesive effects of the hydrogen peroxide (B. Halliwell, J. Gutteridge "Free Radicals in Biology and Medicine", page 416, 1993).

The method described by Herman et Al. (Herman C., Zeiner M.A., Dimmeler S., Arterioscler. Thromb. Vasc. Biol. 17 (12), 3588-82, 1997).

Human endothelial cells of the umbilical vein are prepared according to a standard method. Fresh umbilical veins, just removed, are filled with a solution of collagenase at 0.1% and incubated at 37°C for 5 minutes.

Subsequently the veins are perfused with medium M 199 (GIBCO, Grand Island, NY) pH 7.4 containing 20% of human serum. The cells are collected from the perfusate by centrifugation at 800 rpm and harvested in culture flasks T-75, pretreated with human fibronectin. Cells are then harvested in the medium pH 7.4, containing 20% human serum, low molecular weight sodium heparin (30 mcg/ml), penicillin (100,000 UI/ml) and bovine hypothalamic growth factor (100 ng/ml). The primary confluent monolayers (about 8,000,000 cells/flask) are washed and trypsinized. The cellular suspensions are transferred into each well of a culture plate with 24 hollows and harvested in a thermostat at 37°C at constant humidity (90%), CO_2 = 5%. Only

above dose p.o.,

- Alendronate, dose 100 mg/kg p.o.,
- Nitroxyester of the alendronic acid according to Ex. 4 at the same above dose, p.o.

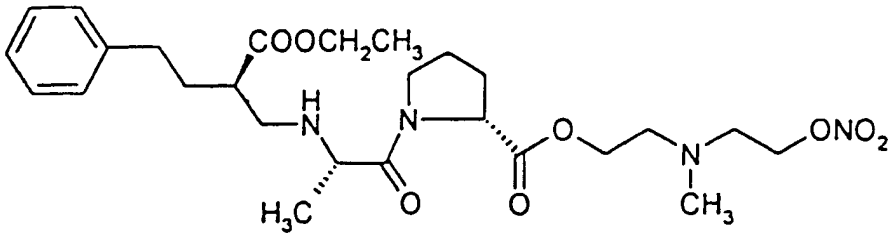
Tacrine and the corresponding nitroxyester obtained according to Ex. 11, have been administered to the rats by subcutaneous route in a physiological solution at the dose of 10 mg/kg.

The animals have been sacrificed 6 hours after the administration. The gastrointestinal mucosa has been removed and inspected. The incidence of the gastrointestinal damage has been evaluated as described in experiment F1.

The results are reported in Table VII and show that the compounds of the invention do not either induce gastric lesions or, in the case, the incidence of said lesions is much lower than that found with the precursor drug.

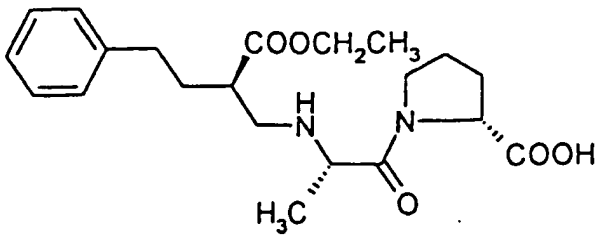
EXAMPLE 16

Synthesis of (S)-1-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline[2-(N-methyl,N'-(2-nitroxy)ethyl)-ammino] ethyl ester of formula



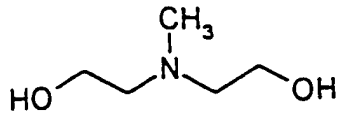
(E-16)

The precursor is enalapril having formula:



(E-16a)

and the precursor of B is N-metil-diethanolamine of formula:



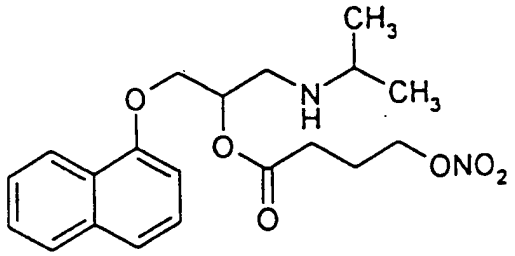
The compound of formula (E-16) is synthetized according to the process described in Example 5. Yield: 19%

Elemental analysis:

Calculated %	C 58,19	H 7,51	N 10,44
Found %	C 58,22	H 7,53	N 10,42

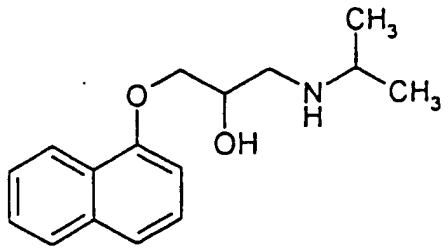
EXAMPLE 17

Synthesis of (4-nitroxy)-butanoic acid 1-[(1-methylethyl) amino]-3-(1-naphthalen oxy)-2-propyl ester of formula



(E-17)

The precursor is propranolol having the following formula:



(E-17a)

and the precursor of B is 4-hydroxy-butanoic acid.

Compound (E-17) is synthetized according to Example 1.

Yield: 25%.

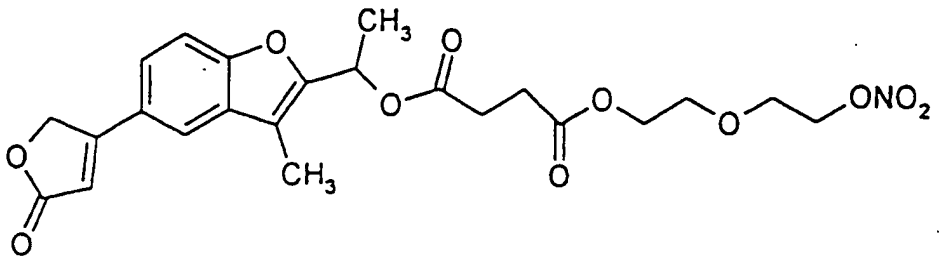
Elemental analysis:

Calculated %	C 61,53	H 6,71	N 7,17
Found %	C 61,58	H 6,74	N 7,15

EXAMPLE 18

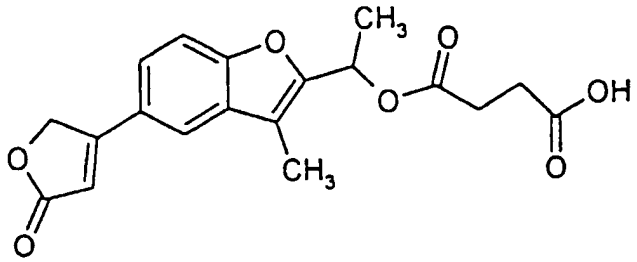
Synthesis of butandioic acid [1-[5-(2,5-dihydro-5-oxo-3-

furanyl)-3-methyl-2-benzofuranyl]ethyl [(2-nitroxy)ethoxy] ethyl diester of formula



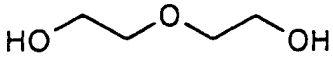
(E-18)

The precursor drug is Benfurodil hemisuccinate having formula:



(E-18a)

and the compound precursor of B is diethylene glycol of formula:

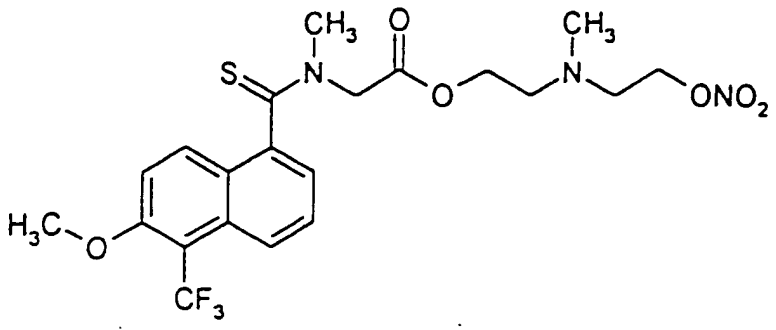


Compound (E-18) is synthetized according to Example 6.
Yield: 16%.
Elemental analysis:

Calculated %	C 56,21	H 5,13	N 2,85
Found %	C 56,26	H 5,10	N 2,90

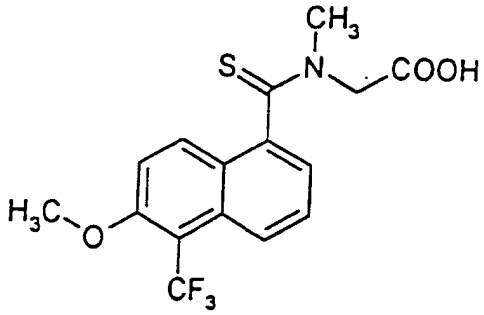
EXAMPLE 19

N-[[6-methoxy-5-(trifluoromethyl)-1-naphtalenyl[thioxomethyl] -N-methylglycine [2-(N-methyl,N'-(2-nitroxy)ethyl)ammino] ethyl ester of formula



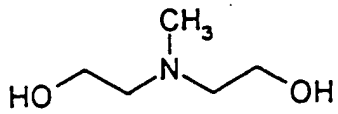
(E-19)

The precursor drug is tolrestat of formula:



(E-19a)

and the precursor of B is N-metil diethanolamine of formula:

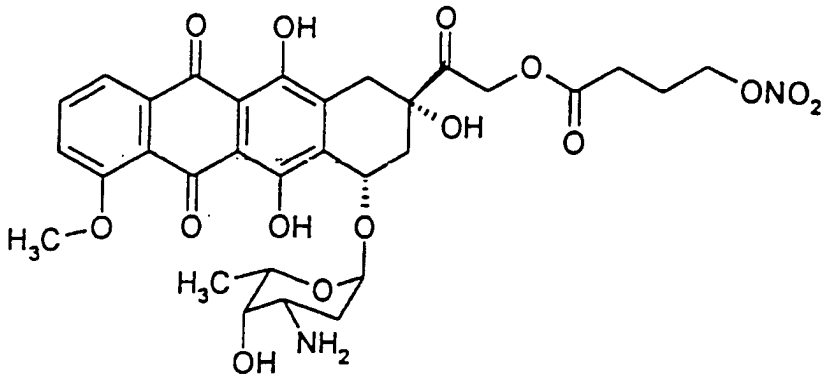


Compound (E-19) was synthetized according to Example 5.
Yield: 12%
Elemental analysis:

Calc. %	C 50,10	H 4,80	N: 8,35	S 6,30	F 11,32
Found %	C 50,15	H 4,82	N 8,30	S 6,25	F 11,34

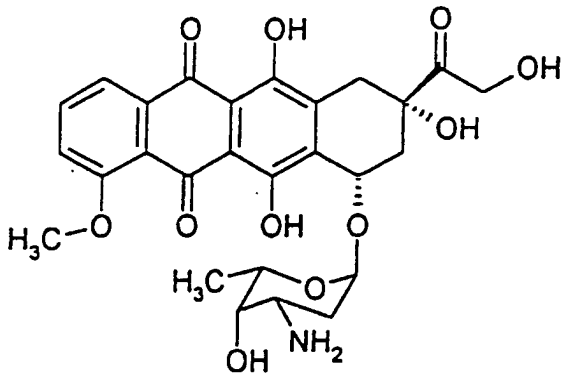
EXAMPLE 20

Synthesis of (8S-cis)-10[(3-amino,2,3,6-tri-deoxy-α-L-lyxo-exopyranosyl)oxy]-7,8,9,10-tetrahydro,6,8,11-trihydroxy-8-[[3-methoxy-4-(4-nitroxy butanoyl-oxy] methyl-oxo]-1-methoxy-5,12-naphtacenedione of formula



(E-20)

The precursor drug is doxorubicin of formula:



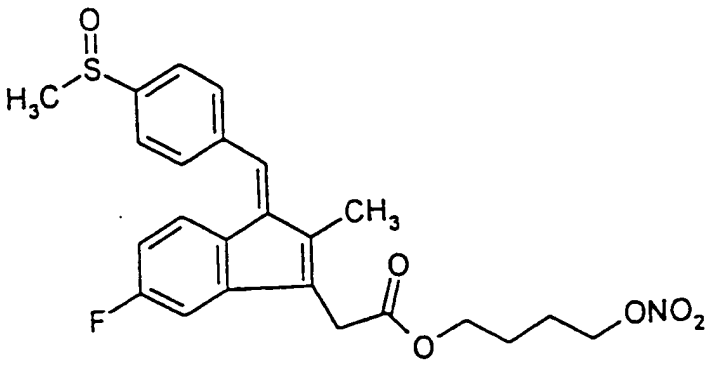
(E-20a)

The compound precursor of B is 4-hydroxy-butyric acid
Compound (E-20) is synthetized according to the process of Example 1. Yield: 12%
Elemental analysis:

Calculated %	C 55,19	H 5,08	N 28,01
Found %	C 55,21	H 5,09	N 28,08

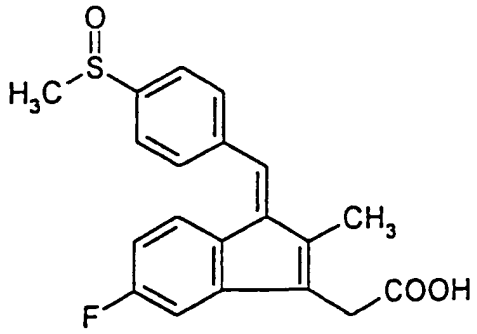
EXAMPLE 21

Synthesis of (Z)-5-fluoro-2-methyl-1-[[4-(methyl sulphinyl)phenyl]methylene]-1H-indene-3-acetic acid (4-nitroxy)butyl ester of formula:



(E-21)

The precursor drug is Sulindac of formula:



(E-21a)

and the precursor of B is 1,4-butandiolo

a) Preparation of cis-5-fluoro-2-methyl-1-[p-(methy sulphinyl) benzyliden]indene-3-acetic acid 4-bromo butil ester

To a solution of sulindac (5.17 g, 14.5 mmole) in dimethylformamide (50 ml) EtONa (1.18 g, 16.4 mmole) is added. The reaction mixture is kept under stirring for one hour, then a solution of 1,4-dibromobutane in dimethylformammide (20 ml) is added.

The reaction mixture is stirred at room temperature for 8 hours, then diluted with ethyl ether and washed with water. The organic phase is dehydrated on sodium sulphate and then evaporated at a reduced pressure. The raw product thus obtained is purified by column chromatography on silica gel, the eluent being n-hexane /ethyl acetate 3/7 (ratio by volume). It is obtained cis-5-fluoro-2-methyl-1-[p-(methylsulfinyl)

benzyliden]indene-3-acetic acid 4-bromobutyl ester.

b) Preparation of cis-5-fluoro-2-methyl-1-[p-(methyl sulphinyl)benzyliden]indene-3-acetic acid 4-(nitroxy)butyl ester

To a solution of cis-5-fluoro-2-methyl-1-[p-(methylsulfinyl) benzyliden]indene-3-acetic acid 4-bromobutyl ester (5.01 g, 10.18 mmole) in acetonitrile (60 ml) silver nitrate is addeed (3.5 g, 20.6 mmole). The reaction mixture is stirred at a temperature of 80°C for 48 hours in the absence of light, then cooled at room temperature and filtered to remove the formed insoluble silver salts and evaporated under a reduced pressure. The residue is purified by column chromatography on silica gel, eluted with n-hexane/ ethyl acetate 3/7 (ratio by volume). After evaporation of the solvent it is obtained (Z)-5-fluoro-2-methyl-1-[[4-(methyl sulphinyl)phenyl]methylene]-1H-indene-3-acetic acid (4-nitroxy)butyl ester (m.p. 93-97). Yield 40%.

Elemental analysis:

Calc. %	C	60.87	H	5,11	F	4,01	N	2,96	S 6,77
Found %	C	60.85	H	5,13	F	3,93	N	2,94	S 6,75

EXAMPLE F8

Example F1 was repeated with three groups of rats (each group of of ten animals), all of them receiving NEM, and orally administered as it follows :

a. control group : the vehicle formed of an aqueous suspension 1% w/v of carboxymethylcellulose,
b. one group (group b - comparative) administered at the same time with 10 mg/Kg (0.034 mmoles/Kg) of diclofenac + 4 mg/Kg (0.034 mmoles/Kg) of N-methyldiethanolamine in the same above vehicle,
c. one group (group c) administered with 15 mg/Kg (0.034 mmoles/Kg) of the ester derivative of diclofenac according to the invention (ref. ex. 14), in the above same vehicle.

The results are reported in Table VIII and show that the mixture administered to group b (comparative), was much less effective in reducing gastric lesions than the group (group c) treated with the derivative according to the invention.

EXAMPLE F9

Antiinflammatory and analgesic activity of 4-(nitrooxy) butanoic acid 4-(N-acetylamino)phenyl ester (NO-paracetamol)

and of the precursor paracetamol.

Foreword

The principal therapeutic effects of NSAIDs derives from their ability to inhibit prostaglandin production ("Goodman & Gilman's, The Pharmacological Basis of Therapeutics" 9th Ed. 1996, McGraw Hill page 620) and the agents are classified on the basis of said principle. Sulindac and paracetamol have different mechanism from most currently used NSAIDS in view of their negligible ability to inhibit prostaglandin production. Both they interact with oxygen free radicals.

Antiinflammatory and analgesic activity have been measured according to carrageenan rat paw edema and acetic acid mouse writhing methods. Rats (male, wistar 100-150 g. and mice (male, LACA, 22-35 g) were used. NO-paracetamol, paracetamol or vehicle were given as carboxymethylcellulose suspension (0.5% w/v) in a volume of 1 mg/Kg.

Carrageenan paw edema

Experiments were conducted as described by Al-Swayeh et al., Brit. J. Pharmacol. 129, 343-350 2000). Hind paw volume was determined by plethysmography before and after 3 h after interplantar carrageenan injection (100 microliter, 2% w/v). The compounds were given intraperitoneally 15 ml prior to carrageenan injection. At the end of the experiment animals were killed by cervical dislocation and exsanguination. The Results shown in Table IX are expressed as % of paw edema inhibition, i.e. the paw volume of the controls (vehicle) subtracted of the paw volume of the treated and the obtained difference divided by the paw volume of the controls.

Acetic acid writhing

Experiments were conducted as described by Moore et al. (Br. J. Pharmacol. 102, 198-202 1990). The compounds were given orally 15 minutes prior to intraperitoneal acetic acid (2% w/v in saline pH 2.7, 10ml/Kg). Mice were transferred immediately to individual observation cages and the number of abdominal constrictions monitored over the following 30 minutes. At the end of the observation period the animals were killed by cervical dislocation and exsanguination. Results are expressed as the number of abdominal costrictions (writhings) per 30 minutes test period, expressed as percentage to those observed

in the control group, and are reported in Table IX.

The results of the Table demonstrate that NO-paracetamol is much more active in both tests than paracetamol.

EXAMPLE F10

Liver safety following administration of NO-paracetamol and paracetamol

Rats received either NO-paracetamol (1.4 g/Kg i.p.) or paracetamol (1.16 g/Kg i.p.) or vehicle (0.9% w/v NaCl containing 20% v/v tween-20). After 6 hours the animals were killed by cervical dislocation, trunk blood collected and plasma analysed for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity, liver glutathione and bilirubin concentration.

Glutathione depletion induced by paracetamol is considered a sign of oxidative stress (B. Halliwell, J. Gutterbridge "Free radicals in biology and medicine" 1993, Clarendon Press, pages 334-335).

The results are reported in Table X and are expressed as the percentage calculated on the corresponding values of the vehicle group (100%).

The results demonstrate that administration of paracetamol causes hepatic damage, as from the values of transaminases AST and ALT, and of bilirubin in respect of those of the controls.

Administration of NO-paracetamol induces much lower increases of AST and ALT, whereas the bilirubin concentration is lower than that in the control groups.

Thus, unlike pracetamol, NO-paracetamol is able to spare the liver, even in conditions of oxidative stress (i.e. hepatic glutathione is similarly depleted with paracetamol and NO-paracetamol).

Table I

Test 1 : Gastric tolerability of drugs representative of the drug classes illustrated in the present invention in animals not treated or treated with NEM (oxidative stress conditions). The % incidence is calculated from the ratio between the number of animals found with gastric lesions and that total of the group.

Compound	dose (mg/Kg) /admin. route	Gastro-enteropathy (% incidence)	
		without NEM	with NEM
carrier		0	0
Indomethacin	7.5/p.o.	0	100
Ambroxol	25/p.o.	0	80
Mesalamine	750/i.c.	0	60
Alendronate	15/p.o.	0	90
Tacrine	1/s.c.	0	100
Omeprazol	30/s.c.	0	0
Misoprostol	0.5/s.c.	0	0

p.o. = per os; i.c. = by intracolonic route;
s.c. = by subcutaneous route.

Table II

Test 2 : Inhibition of apoptosis (DNA fragmentation) induced by CIP in the endothelial cells in the presence of compounds representative of the drug classes illustrated in the present invention.	
Compound	Apoptosis % with respect to the controls treated only with CIP
Indomethacin	95
Paracetamol	120
Clopidogrel	110
Salbutamol	90
Ambroxol	70
Alendronate	160
Diphylline	95
Cetirizine	115
Enalapril	80
Nicotinamide	98
Doxorubicin	94
Acyclovir	95
Mesalamine	74
Tacrine	90
Simvastatin	72
Omeprazol	90

Table III

Test 5 : Screening of the effectiveness of the listed substances to inhibit radical production induced by Fe^{II}	
Compound	% Radical inhibition from Fe^{II}
blank	0
N-methyldiethanolamine	0
Diethylenglycol	0
1,4-Butandiol	0
Thiodiethyleneglycol	0

Table IV

Test 3 : Gastric tolerability (gastrointestinal damage incidence), hepatic (GPT dosage, glutamic-pyruvic transaminase), and cardiovascular (blood pressure) of some compounds representative of the drug classes illustrated in the present invention under conditions of endothelial trouble induced by L-NAME.
The results relating to the blood pressure and GPT are expressed as % values compared with those found in animals treated with the only carrier, without L-NAME.

Compound	dose mg/Kg /administ. route	Blood pressure %		GPT %		Gastroenteropathy %	
		without L-NAME	with L-NAME	without L-NAME	with L-NAME	without L-NAME	with L-NAME
Carrier		100	152	100	155	0	30
Paracetamol	300/i.p.	108	155	180	500	20	90
Doxorubicin	1/i.p.	120	145	195	360	30	100
Simvastatin	50/p.o.	85	148	122	220	0	60
Omeprazol	30/s.c.	100	150	100	160	0	10
Misoprostol	0.5/s.c.	100	142	100	160	0	5

Table V

Test 4A: Screening of the effectiveness of the listed substances to inhibit erythrocyte haemolysis induced by cumene hydroperoxide	
Compound	% Haemolysis inhibition
N-Methyldiethanolamine	54.4
Diethylenglycol	33.4
Thiodiethylenglycol	26
1,4-Butandiol	17.4
Butanol	10.5
Diethanolamine	2.5

Table VI

Experiment F6: Apoptosis inhibition (DNA fragmentation) induced in endothelial cells by hydrogen peroxide, by precursors representative of the drug classes described in the present invention and of the corresponding derivatives of the invention.	
Compound	Apoptosis % (respect to the controls treated only with CIP)
Carrier	0
Diclofenac (comp.)	15
Diclofenac nitroxyester Es. 14	72
Ambroxol (comp.)	25
Ambroxol nitroxyester Ex. 3	50
Alendronate (comp.)	18
Alendronate nitroxyester Ex. 4	54
Tacrine (comp.)	8
Tacrine nitroxyester Ex. 11	73

Table VII

Experiment F7: screening of the gastric tolerability of the derivatives according to the present invention compared with that of the precursor drugs		
Treatment	dose mg/kg	Gastropathy % incidence
Carrier	-	0
Diclofenac (comp.)	20 p.o.	70
Diclofenac nitroxyester Es. 14	20 p.o.	0
Ambroxol (comp.)	100 p.o.	60
Ambroxol nitroxyester Ex. 3	100 p.o.	10
Alendronate (comp.)	100 p.o.	100
Alendronate nitroxyester Ex. 4	100 p.o.	10
Tacrine (comp.)	10 p.o.	60
Tacrine nitroxyester Ex. 11	10 s.c.	20

Table VIII

Test on gastric tolerability following oral administration of NEM (Ex. F8)		
groups	dose mg/Kg p.o.	Gastropathy % incidence
controls	-	-
group b - comparative mixture diclofenac (A) + N-methyldiethanolamine (B)	10(A) + 4(B)	50
group c diclofenac derivative according to the invention (ref. ex. 14)	14	20

Table IX

Antiinflammatory and analgesic activity of NO-paracetamol and paracetamol.		
Treatment	Antiinflammatory activity % paw edema inhibition	Analgesic activity % writhing inhibition
vehicle	-	-
paracetamol	34	40
NO-paracetamol	69	490

Table X

Liver safety assayed by AST (aspartate aminotransferase) ALT (alanine aminotransferase), glutathione and bilirubin concentration in animals treated with NO-paracetamol and paracetamol. The values given in the Table are expressed as % to the corresponding of the control group.				
Treatment	AST %	ALT %	Glutathione %	Bilirubin %
vehicle	100	100	100	100
paracetamol	330	171	52	200
NO-paracetamol	160	57	49	136

CLAIMS

1. Compounds or their salts having the following general formula (I):

$$A—B—N(O)_s \qquad (I)$$

wherein:

s is an integer equal to 1 or 2, preferably s = 2;

A = $R—T_1$-, wherein

R is the drug radical and

$T_1 = (CO)_t$ or $(X)_{t'}$, wherein X = O, S, NR_{1C}, R_{1C} is H or a linear or branched alkyl, having from 1 to 6 carbon atoms, or a free valence, t and t' are integers and equal to zero or 1, with the proviso that t = 1 when t' = 0; t = 0 when t' = 1;

B = -$T_B—X_2—O$- wherein

T_B = (CO) when t = 0, T_B = X when t' = 0, X being as above defined;

X_2, bivalent radical, is such that the corresponding precursor of B does not meet test 5 and meets test 4A; said precursor having formula -$T_B—X_2$-OH, wherein T_B = (CO) and t = 0, the free valence of T_B is saturated with:

-OZ wherein Z = H or R_{1a}, R_{1a} being linear or branched when possible C_1-C_{10} alkyl, preferably C_1-C_5, or with -Z^I-N-Z^{II}, Z^I and Z^{II} being equal or different from each other, having the Z values, when T_B = X and t' = 0, the free valence of T_B is saturated with H;

with the proviso that:

the drug A = $R—T_1$-, wherein the free valence is saturated as hereinafter mentioned:

- when t' = O with:
  - O-Z wherein Z = H or R_{1a} as above defined, or with
  - Z^I-N-Z^{II}, Z^I and Z^{II} being as above defined,
- when t = 0 with X-Z, wherein X and Z as above defined,

is such as to meet at least one of tests 1-3;

- wherein test 1 (NEM) is a test in vivo carried out on

four groups of rats (each formed by 10 rats), the controls (two groups) and the treated (two groups) of which one group of the controls and one group of the treated respectively are administered with one dose of 25 mg/kg s.c. of N-ethylmaleimide (NEM), the controls being treated with the carrier and the treated groups with the carrier + the drug of formula $A = R\text{-}T_1\cdot$ wherein the free valence is saturated as above indicated, administering the drug at a dose equivalent to the maximum one tolerated by the rats that did not receive NEM, i.e. the highest dose administrable to the animal at which there is no manifest toxicity, i.e. such as to be symptomatologically observable; the drug complies with test 1, i.e. the drug can be used to prepare the compounds of general formula (I), when the group of rats treated with NEM + carrier + drug shows gastrointestinal damages, or in the group treated with NEM + carrier + drug are observed gastrointestinal damages greater than those of the group treated with the carrier, or of the group treated with the carrier + drug, or of the group treated with the carrier + NEM;

- wherein test 2 (CIP) is a test in vitro wherein human endothelial cells from the umbilical vein are harvested under standard conditions, then divided into two groups (each group replicated five times), of which one is treated with a mixture of the drug 10^{-4} M concentration in the culture medium, the other group with the carrier; then cumene hydroperoxide (CIP) having a 5 mM concentration in the culture medium is added to each of the two groups; the drug meets test 2, i.e. the drug can be used to prepare the compounds of general formula (I), when a statistically significant inhibition of the apoptosis (cellular damage) induced by CIP is not obtained with $p< 0.01$ with respect to the group treated with the carrier and CIP;
- wherein test 3 (L-NAME) is a test in vivo carried out on four groups of rats (each group formed by 10 rats) for 4 weeks and receiving drinking water, the controls (two groups) and the treated (two groups), of which one group of the controls and of the treated respectively receives

in the above 4 weeks drinking water added of N-ω-nitro-L-arginine methyl ester (L-NAME) at a concentration of 400 mg/litre, the controls in the 4 weeks being administered with the carrier and the treated in the 4 weeks with the carrier + the drug, administering the carrier or the drug + carrier once a day, the drug being administered at the maximum dose tolerated by the group of rats not pretreated with L-NAME, i.e., the highest dose administrable to animals at which no manifest toxicity appears, i.e. such as to be symptomatologically observable; after the said 4 weeks, the water supply is stopped for 24 hours and then sacrified, determining the blood pressure 1 hour before sacrifice, and after sacrifice of the rats determining the plasma glutamic pyruvic transaminase (GPT) after sacrifice, and examining the gastric tissue; the drug meets test 3, i.e. the drug can be used to prepare the compounds of general formula (I), when in the group of rats treated with L-NAME + carrier + drug, greater hepatic damages (determined as higher values of GPT) and/or gastric and/or cardiovascular damages (determined as higher values of blood-pressure) are found in comparison respectively with the group treated with the carrier alone, or with the group treated with the carrier + drug, or with the group treated with the carrier + L-NAME;

- wherein test 4A which must be met by the compound precursor of B is a test in vitro wherein a portion of an erythrocite suspension formerly kept at 4°C for 4 days, said erythrocyte isolated by standard procedures from Wistar male rats and suspended in a physiological solution buffered at pH 7.4 with phosphate buffer, is centrifuged at 1000 rpm for 5 minutes and 0.1 ml of the centrifuged erythrocytes are diluted with sodium phosphate buffer pH 7.4 at 50 ml; aliquots of 3,5 ml each (No. 5 samples) are taken from said diluted suspension and incubated at 37°C in the presence of cumene hydroperoxide at a concentration 270 µM and the suspension turbidity determined at 710 nm at intervals of 30 minutes to establish the time (Tmax) at which occurs the maximum turbidity, that corresponds to the maximum amounts of cells lysed by cumene hydroperoxide

(haemolysis assumed to be = 100%); then alcoholic solutions of the compounds precursors of B are added to 3.5 ml aliquots of the diluted suspension of centrifuged erythrocytes (tests carried out on 5 samples for each precursor of B assayed) in order to have a final concentration 2 mM of the precursor of B and then the resulting suspension preincubated for 30 minutes, cumene hydroperoxide is added in a quantity to have the same above indicated final concentration and at Tmax is determined the percentage of haemolysis inhibition in the sample from the ratio, multiplied by 100, between the absorbance of the sample containing the erythrocytes, the precursor of B and cumene hydroperoxide respectively and that of the sample containing the erythrocytes and cumene hydroperoxide; the precursors of B meet the test if they inhibit the haemolysis induced by cumene hydroperoxide by a percentage > 15%;

- wherein test 5 which must not be met by the precursor compound of B is an analytical determination carried out by adding aliquots of 10^{-4} M methanol solutions of the precursor of B or B_1 or of C = $-T_C-Y-H$, having the free valence saturated as above indicated, to a solution formed by admixing a 2 mM solution of deoxyribose in water with 100 mM of phosphate buffer and 1 mM of the salt $Fe^{II}(NH_4)_2(SO_4)_2$; after having thermostatted the solution at 37°C for one hour, are added, in the order, aliquots of aqueous solutions of trichloroacetic acid 2.8% and of thiobarbituric acid 0.5 M, heating is effected at 100°C for 15 minutes and the absorbance of the tested solutions is then read at 532 nm; the inhibition induced by the precursor of B or B_1 or C = $-T_C-Y-H$ in the confront of radical production by FE^{II} is calculated as a percentage by means of the following formula:

$$(1 - A_s/A_c)X100$$

wherein A_s and A_c are respectively the absorbance values of the solution containing the tested compound and the iron salt and that of the solution containing only the iron salt; test 5 is met when the inhibition percentage as above defined of the B precursor is higher than or equal

to 50%;

provided that in formula (I) when X_2 of B is a linear or branched C_1 - C_{20} alkylene or a cycloalkylene having from 5 to 7 carbon atoms optionally substituted,

the drugs of formula A = R-T_1, with the free valence saturated as above described, used in the compound of formula (I), has not to belong to the following classes: drugs for use in incontinence, antithrombotic drugs (ACE inhibitors), prostaglandins, antiinflammatory drugs (NSAIDS and corticosteroids) but not excluding from the antiinflammatory NSAIDS paracetamol and sulindac.

2. Compounds according to claim 1, wherein in the formula -T_B-X_2-O- of the precursor compound of B which meets test 4A and does not meet test 5, X_2 is equal to the R_{1B}-X-R_{2B} radical wherein R_{1B} and R_{2B}, equal to or different from each other, are linear or branched C_1-C_6 alkylenes, or X_2 is a radical wherein two alkylene chains C_1-C_4, preferably C_1-C_2, are linked to non adjacent positions of a central ring having 4 or 6 atoms, preferably 5 or 6 atoms, said ring being an unsaturated cycloaliphatic ring, or a saturated or aromatic eterocyclic ring, containing one or two heteroatoms, equal or different, selected from O, S,N. By unsaturated cycloaliphatic ring it is meant a ring that has not an aromatic character according to the Hückel's rule.

3. Compounds according to claims 1 and 2, wherein the precursor compounds of B are:

1,4-butandiol: HO-$(CH_2)_4$-OH,

6-hydroxyhexanoic acid: HO-$(CH_2)_5$-COOH,

4-hydroxybutyric acid: HO-$(CH_2)_3$-COOH,

N-methyldiethanolamine: HO-$(CH_2)_2$-N(CH_3)-$(CH_2)_2$-OH,

diethylenglycol: HO-$(CH_2)_2$-O-$(CH_2)_2$-OH,

thiodiethylenglycol: HO-$(CH_2)_2$-S-$(CH_2)_2$-OH; 1,4 dioxane-2,6-dimethanol, tetrahydropyrane-2,6-dimethanol, 4H pyrane-2,6-dimethanol, tetrahydrothiopyrane-2,6-dimethanol, 1,4-dithiane-2,6-dimethanol, cyclohexene-1,5-dimethanol, thiazole-2,5-dimethanol, thiophene-2,5-dimethanol, oxazole-2,5-dimethanol, preferably N-methyldiethanolamine, diethylenglycol,

thiodiethylenglycol.

4. Compounds according to claims 1-3, wherein the precursor drugs of the compounds of formula (I) are selected from the following: anti-inflammatory, analgesic drugs, bronchodilators and drugs active on the cholinergic system, expectorant-mucolytics, antiasthmatic-antiallergic drugs, antihistaminic drugs, ACE-inhibitors, beta-blockers, antithrombotic drugs, vasodilators, antidiabetic, atitumoral, antiulcer drugs, antihyperlipidemic drugs, antibiotics, antiviral drugs, bone resorption inhibitors, antidementia drugs.

5. Compounds according to claim 4, wherein the precursor drugs are selected from the following:
anti-inflammatory drugs: aceclofenac, acemetacin, acetylsalicylic acid, 5-aminoacetylsalicylic acid, alclofenac, alminoprofen, amfenac, bendazac, bermoprofen, α-bisabolol, bromfenac, bromosaligenin, bucloxic acid, butibufen, carprofen, cinmetacin, clidanac, clopirac, sodium diclofenac, diflunisal, ditazol, enfenamic acid, etodolac, etofenamate, felbinac, fenbufen, fenclozic acid, fendosal, fenoprofen, fentiazac, fepradinol, flufenamic acid, flunixin, flunoxaprofen, flurbiprofen, glucametacin, glycol salicylate, ibuprofen, ibuproxam, indomethacin, indoprofen, isofezolac, isoxepac, isoxicam, ketoprofen, ketorolac, lornoxicam, loxoprofen, meclofenamic acid, mefenamic acid, meloxicam, mesalamine, metiazinic acid, mofezolac, naproxen, niflumic acid, olsalazine, oxaceprol, oxaprozin, oxyphenbutazone, parsalmide, perisoxal, phenyl acetylsalicylate, pyrazolac, piroxicam, pirprofen, pranoprofen, protizinic acid, salacetamide, salicilamide O-acetic acid, salicylsulphuric acid, salsalate, sulindac, suprofen, suxibuzone, tenoxicam, tiaprofenic acid, tiaramide, tinoridine, tolfenamic acid, tolmetin, tropesin, xenbucin, ximoprofen, zaltoprofen, zomepirac, tomoxiprol;
analgesic drugs: acetaminophen, acetaminosalol, aminochlorthenoxazin, acetylsalicylic 2-amino-4-picoline acid, acetylsalicylsalicylic acid, anileridine, benoxaprofen benzylmorphine, 5-bromosalicylic acetate acid, bucetin, buprenorphine, butorphanol, capsaicine,

cinchophen, ciramadol, clometacin, clonixin, codeine, desomorphine, dezocine, dihydrocodeine, dihydromorphine, dimepheptanol, dipyrocetyl, eptazocine, ethoxazene, ethylmorphine, eugenol, floctafenine, fosfosal, glafenine, hydrocodone, hydromorphone, hydroxypethidine, ibufenac, p-lactophenetide, levorphanol, meptazinol, metazocine, metopon, morphine, nalbuphine, nicomorphine, norlevorphanol, normorphine, oxycodone, oxymorphone, pentazocine, phenazocine, phenocoll, phenoperidine, phenylbutazone, phenylsalicylate, phenylramidol, salicin, salicylamide, tiorphan, tramadol, diacerein, actarit;

broncodilators and drugs active on the cholinergic system: acefylline, albuterol, bambuterol, bamifylline, bevonium methyl sulphate, bitolterol, carbuterol, clenbuterol, chlorprenaline, dioxethedrine, difylline, ephedrine, epinephrine, eprozinol, etafredine, ethylnorepinephrine, etofylline, fenoterol, flutoprium bromide, hexoprenaline, ipratropium bromide, isoetharine, isoprotenerol, mabuterol, metaproterenol, oxybutinyn, oxitropium bromide, pirbuterol, procaterol, protokylol, proxyphylline, reproterol, rimiterol, salmeterol, soterenol, terbutaline, 1-teobromineacetic acid, tiotropium bromide, tretoquinol, tulobuterol, zaprinast, cyclodrine, NS-21, 2-hydroxy-2,2-diphenyl-N-(1,2,3,6-tetrahydro-pyridin-4-ylmethyl)acetamide;

expectorant/mucolytic drugs: acetil-cysteine, ambroxol, bromhexine, carbocysteine, domiodol, erdosteine, ferulic acid, guaiacol, guaifenesin, iodinated glycerol, letosteine, mecysteine hydrochloride, mesna, sobrerol, stepronin, terpin, tiopronin;

antiasthmatic/antiallergic antihistaminic drugs: acrivastine, alloclamide, amlexanox, cetirizine, clobenzepam, chromoglycate, chromolyn, epinastine, fexofenadine, formoterol, histamine, hydroxyzine, levocabastine, lodoxamide, mabuterol, metron s, montelukast, nedocromil, repirinast, seratrodast, suplatast tosylate, terfenadine, tiaramide, urushiol, bromhexine;

ACE-inhibitors: alacepril, benazepril, captopril, ceronapril, cilazapril, delapril, enalapril,

enalaprilat, fosinopril, imidapril, lisinopril, losartan, moveltipril, naphthopidil, perindopril, quinapril, ramipril, spirapril, temocapril, trandolapril, urapidil;

beta-blockers: acebutolol, alprenolol, amosulalol, arotinolol, atenolol, betaxolol, bevantolol, bucumolol, bufetolol, bufuralol, bunitrolol, bupranolol, butofilol, carazolol, carteolol, carvedilol, celiprolol, cetamolol, dilevalol, epanolol, esmolol, indenolol, labetalol, mepindolol, metipranolol, metoprolol, moprolol, nadolol, nadoxolol, nebivolol, nifenalol, nipridalol, oxprenolol, penbutolol, pindolol, practolol, pronethalol, propranolol, sotalol, sulfinalol, talinolol, tertatolol, tilisolol, timolol, toliprolol, xibenolol;

antithrombotic and vasoactive drugs: acetorphan, acetylsalicylic acid, argatroban, bamethan, benfurodil hemisuccinate, benziodarone, betahistine, brovincamine, bufeniode, citicoline, clobenfurol, clopidogrel, cyclandelate, dalteparin, dipyridamole, droprenilamine, enoxaparin, fendiline, ifenprodil, iloprost, indobufen, isbogrel, isoxsuprine, heparin, lamifiban, midodrine, nadroparin, nicotinyl alcohol, nylidrin, ozagrel, perhexiline, phenylpropanolamine, prenylamine, papaveroline, reviparin sodium salt, ridogrel, suloctidil, tinofedrine, tinzaparin, triflusal, xanthinol niacinate;

antidiabetic drugs: acarbose, carbutamide, glibornuride glybuthiazol(e), miglitol, repaglinide, troglitazone, 1-butyl-3-metanyl-urea, tolrestat, nicotinamide;

antitumoral drugs: ancitabine, anthramycin, azacitidine, azaserine, 6-azauridine, bicalutamide, carubicin, carzinophilin, chlorambucil, chlorozotocin, cytarabine, daunorubicin, defosfamide, demecolcine, denopterin, 6-diazo-5-oxo-L-norleucine, docetaxel, doxifluridine, doxorubicin, droloxifene, edatrexate, eflornithine, enocitabine, epirubicin, epitiostanol, etanidazole, etoposide, fenretinide, fludarabine, fluorouracil, gemcitabine, hexestrol, idarubicin, lonidamine, mannomustine, melphalan, menogaril, 6-

mercaptopurine, methotrexate, mitobronitol, mitolactol, mitomycins, mitoxantrone, mopidamol, mycophenolic acid, ninopterin, nogalamycin, paclitaxel, pentostatin, pirarubicin, piritrexim, plicamycin, podopillic acid, porfimer sodium, porfiromycin, propagermanium, puromycin, ranimustine, retinoic acid, roquinimex, streptonigrin, streptozocin, teniposide, tenuazonic acid, thiamiprine, thioguanine, tomudex, topotecan, trimetrexate, tubercidin, ubenimex, vinblastine, vincristine, vindesine, vinorelbine, zorubicin;

antiulcer drugs: acetamidocaproic acid, arbaprostil, cetraxate, cimetidine, ecabet, enprostil, esaprazole, irsogladine, misoprostol, omeprazole, ornoprostil, pantoprazole, plaunotol, rioprostil, rosaprostol, rotraxate, sofalcone, trimoprostil;

anti-hyperlipidemic drugs: atorvastatin, cilastatin, dermostatin, fluvastatin, lovastatin, mevastatin, nystatin, pentostatin, pepstatin, privastatin sodium, simvastatin;

antibiotics: amdinocillin, amoxicillin, ampicillin, apalcillin, apicycline, aspoxicillin, azidamfenicol, azidocillin, azlocillin, aztreonam, benzoylpas, benzyl penicillinic acid, biapenem, bicozamycin, capreomycin, carbenicillin, carindacillin, carumonam, cefaclor, cefadroxil, cefamandole, cefatrizine, cefazedone, cefazolin, cefbuperazone, cefclidin, cefdinir, cefditoren, cefepime, cefetamet, cefixime, cefmenoxime, cefmetazole, cefminox, cefodizime, cefonicid, cefoperazone, ceforanide, cefotaxime, cefotetan, cefotiam, cefoxitin, cefozopran, cefpimizole, cefpiramide, cefpirome, cefprozil, cefroxadine, cefsulodin, ceftazidime, cefteram, ceftezole, ceftibuten, ceftiofur, ceftizoxime, ceftriaxone, cefuroxime, cefuzonam, cephacetrile sodium, cephalexin, cephaloglycin, cephaloridine, cephalosporin C, cephalothin, cephapirin sodium, cephradine, chloramphenicol, chlortetracycline, cinoxacin, cyprofloxacin, clavulanic acid, clometocillin, cloxacillin, cyclacillin, cycloserine, demeclocycline, dicloxacillin, epicillin, fenbecillin, flomoxef, floxacilli-

n, hetacillin, imipenem, lenampicillin, loracarbef, lymecycline, mafenide, meclocycline, meropenem, metampicillin, methacycline, methicillin sodium, mezlocillin, minocycline, moxalactam, mupirocin, myxin, negamycin, novobiocin, oxacillin, panipenem, penicillin G potassium salt, penicillin N, penicillin O, penicillin V, phenethicillin potassium salt, pipacycline, piperacillin, pirlimycin, porfiromycine, propicillin, quinacillin, ritipenem, rolitetracycline, sancycline, sedecamycine, spectinomycin, sulbactam, sulbenicillin, temocillin, tetracycline, ticarcillin, tigemonam, tubercidin, azithromycin, clarithromycin, dirithromycin, enviomycin, erythromycin, josamycin, midecamycin, miokamycin, oleandomycin, rifabutin, rifamide, rifamycin, rifaximin, rokitamycin, spiramycin, troleandromycin, viomycin, virginiamycin;

amikacin, apramycin, arbekacin, dibekacin, dihydrostreptomycin, fortimicins, gentamicin, micronomicin, neomycin, netilmicin, paromomycin, ribostamycin, sisomicin, spectinomycin, streptomicin, tobramycin, trospectomycin; bacampicillin, cefcapene pivoxil, cefpodoxime proxetil, panipenem, pivampicillin, pivcefalexin, sultamicillin, talampicillin;

carbomycin, clindamycin, lincomycin, mikamycin, rosaramicin, ciprofloxacin, clinafloxacin, difloxacin, enoxacin, enrofloxacin, fleroxacin, flumequine, grepafloxacin, lomefloxacin, nadifloxacin, nalidixic acid, norfloxacin, ofloxacin, pazufloxacin, pefloxacin, pipemidic acid, piromidic acid, rufloxacin, sparfloxacin, tosufloxacin, trovafloxacin, clomocycline, guamecycline, oxytetracycline, nifurpirinol, nifurprazine;

p-aminosalicylic acid, p-aminosalicylic acid hydrazide, clofazimine, deoxydihydrostreptomycin, ethambutol, glyconiazide, isoniazid, opiniazide, phenyl aminosalicylate, rifampin, rifapentine, salinazid, 4-4'-sulfynyldianiline,

acediasulfone, dapsone, succisulfone, p-sulfanilylbenzyl amine, thiazolsulfone, acetyl sulfamethoxypyrazine, mafenide, 4'-(methylsulfamoyl)sulfanilanilide,

salazosulfadimidine, sulfabenzamide, sulfacetamide, sulfachlorpyridazine, sulfachrysoidine, sulfacytine, sulfadiazine, sulfadicramide, sulfadimethoxine, sulfadoxine, sulfaethidole, sulfaguanidine, sulfaguanole, sulfalene, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethomidine, sulfamethoxazole, sulfamethoxypyridazine, sulfamethylthiazole, sulfametrole, sulfamidochrysoidine, sulfamoxole, sulfanilamide, 2-p-sulfanilylanilinoethanol, N^4-sulfanilylsulfanilamide, sulfanilylurea, N-sulfanilyl-3,4-xylamide, sulfaperine, sulfaphenazole, sulfaproxyline, sulfapyrazine, sulfapyridine, sulfasomizole, sulfasymazine, sulfathiazole, sulfathiourea, sulfisomidine, sulfisoxazole, 4-sulfanilamido salicylic acid; negamycin, carumonan, cloxyquin, nitroxoline, arginine, metronidazole;

antiviral drugs: acyclovir, amantadine, cidofovir, cytarabine, didanosine, dideoxyadenosine, edoxudine, famciclovir, floxuridine, ganciclovir, idoxuridine, indanavir, kethoxal, lamivudine, MADU, penciclovir, podophyllotoxin, ribavirin, rimantadine, saquinavir, sorivudine, stavudine, trifluridine, valacyclovir, vidarabine, xenazoic acid, zalcitabine, zidovudine;

bone resorption inhibitors: alendronic acid, butedronic acid, etidronic acid, oxidronic acid, pamidronic acid, risedronic acid;

antidementia drugs: amiridine, lazabemide, mofegiline, salbeluzol, oxiracetam, ipidacrine, nebracetam, tacrine, velnacrine.

6. Compounds according to claims 4 and 5, wherein the precursor drugs are selected from the following:

anti-inflammatory drugs: acetylsalicylic acid, 5-aminoacetylsalicylic acid, carprofen, diclofenac sodium, diflunisal, etodolac, flufenamic acid, flunixin, flurbiprofen, ibuprofen, indomethacin, indoprofen, ketoprofen, ketorolac, lornoxicam, loxoprofen, meclofenamic acid, mefenamic acid, meloxicam, mesalamine, naproxen, niflumic acid, olsalazine, piroxicam, salsalate, sulindac, suprofen, tenoxicam, tiaprofenic acid, tolfenamic acid, tolme-

tin, zomepirac, tomoxiprol;

analgesic drugs: acetaminophen, acetylsalicylsalicylic acid, benoxaprofen, buprenorphine, butorphanol, capsaicin, diacereine, dihydrocodeine, ethylmorphine, eugenol, phenylbutazone, meptazinol, morphine, nalbuphine, pentazocine, thiorphan, tramadol, actarit;

bronchodilators and drugs active on the cholinergic system: albuterol, carbuterol, clenbuterol, diphylline, etophylline, fenoterol, ipratropium bromide, metaproterenol, oxybutynin pirbuterol, salmeterol, terbutaline, tiotropium bromide, zaprinast, cyclodrine, NS-21, 2-hydroxy-2,2-diphenyl-N-(1,2,3,6-tetrahydro-pyridin-4-ylmethyl) acetamide;

expectorant/mucolytic drugs: acetyl-cysteine, ambroxol, bromexine, carbocysteine, guaiacol, ferulic acid, mecysteine hydrochloride, sobrerol;

antiasthmatic/antiallergic antihistaminic drugs: cetirizine, chromoglycate, histamine, levocabastine, lodoxamide, montelukast, terfenadine, bromhexine.

ACE-inhibitors: captopril, enalapril, lisinopril, losartan, ramipril;

beta blockers: alprenolol, atenolol, bupranolol, labetalol, metipranolol, metoprolol, pindolol, propranolol, timolol;

antithrombotic and vasoactive drugs: acetylsalicylic acid, acetorphan, argatroban, clopidogrel, dalteparin, dipyridamole, enoxaparin, heparin, iloprost, midodrine, ozagrel, phenylpropanol amine, trifusal;

antidiabetic drugs: tolrestat, nicotinamide;

antitumoral drugs: anthramycin, daunorubicin, doxorubicin, epirubicin, fluorouracil, methotrexate, vinblastine;

antiulcer drugs: cimetidine, omeprazole, pantoprazole;

antihyperlipidemic drugs: lovastatin, pravastatin sodium, simvastatin;

antibiotic drugs: amoxicillin, ampicillin, aztreonam, biapenem, carbenecillin, cefaclor, cefadroxil, cefamandole, cefatrizine, cefoxitin, clavulanic acid,

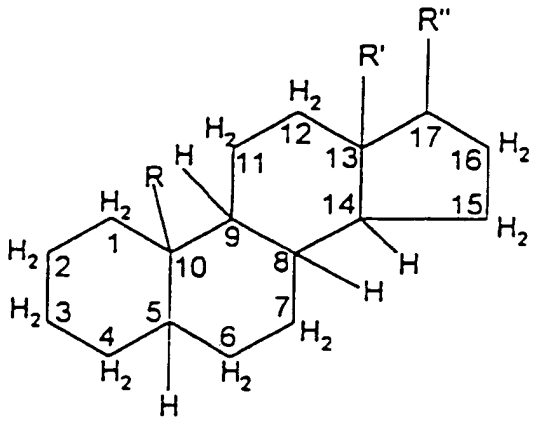
dicloxacillin, imipenem, meclocycline, methacycline, moxalactam, panipenem, sulbactam, azithromycin, erythromycin, josamycin, miokamycin, rifabutine, rifamide, rifamycin, gentamicin, paromomycin, sisomicin, bacampicillin, carbomycin, clindamycin, ciprofloxacin, clinafloxacin, difloxacin, enrofloxacin, lomefloxacin, nadifloxacin, norfloxacin, ofloxacin, pipemidic acid, apicycline, clomocycline, oxytetracycline, nifurpirinol, nifurprazine, isoniazid, rifampin, rifapentine, dapsone, thiazolsulfone, sulfamethoxazole, sulfamoxole, metronidazole, arginine;

antiviral drugs: acyclovir, famciclovir, ganciclovir, penciclovir, ribavirin, vidarabine, zidovudine;

bone resorption inhibitors: alendronic acid, etidronic acid, pamidronic acid;

antidementia drugs: oxiracetam, tacrine, velnacrine.

7. Compounds according to claims 1-3 wherein the precursor drugs are steroid compounds wherein A = R- having the following structure:

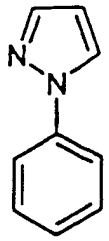


(S-I)

wherein in substitution of the hydrogens of the CH groups or of the two hydrogens of the CH_2 groups mentioned in the general formula, the following substituents can be present:

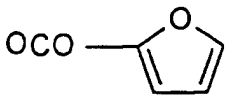
in position 1-2: there may be a double bond;

in position 2-3: there may be the following substituent:



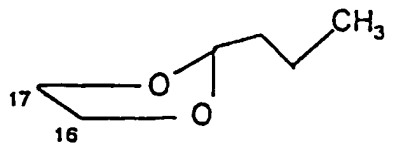
(S-II)

in position 2: there may be Cl, Br;
in position 3: there may be CO, $-O-CH_2-CH_2-Cl$, OH;
in position 3-4: there may be a double bond;
in position 4-5: there may be a double bond;
in position 5-6: there may be a double bond;
in position 5-10: there may be a double bond;
in position 6: there may be Cl, F, CH_3, -CHO;
in position 7: there may be Cl, OH;
in position 9: thre may be Cl, F;
in position 11: there may be OH, CO, Cl, CH_3;
in position 16: there may be CH_3, OH, $=CH_2$:
in position 17: there may be OH, CH_3, $OCO(O)_{ua}(CH_2)_{va}CH_3$, C≡CH or

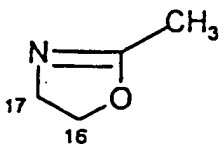


(S-III)

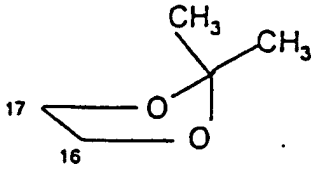
wherein ua is an integer equal to 0 or 1, va is an integer from 0 to 4;
in position 16-17: there may be the following groups:



(S-IVa)



(S-IVb)



(S-IVc)

R and R', equal to or different from each other, can be hydrogen or linear or branched alkyls from 1 to 4 carbon atoms, preferably R = R' = CH_3;

R" is $-(CO-L)_t-(L)_{t2}-(X_O^I)_{t1}-$

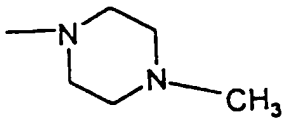
wherein t, t1 and t2 are integers equal to or different from each other, equal to 0 or 1, with the proviso that when t = 0 t2 = 1 and when t = 1 t2 = 0, and that t and t1, or t2 and t1, cannot contemporaneously be equal to 0 when A does not contain -OH groups;

the bivalent bridging group L is selected from:

$(CR_4R_5)_{na}(O)_{nb}(CR_4R_5)_{n'a}(CO)_{n'b}(O)_{n''b}(CO)_{n'''b}(CR_4R_5)_{n''a}$

wherein na, n'a, and n''a, equal to or different from each other, are integers from 0 to 6, preferably 1-3; nb, n'b, n''b and n'''b, equal to or different from each other, are integers equal to 0 or 1; R_4, R_5, equal to or different from each other, are selected from H, linear or branched alkyl from 1 to 5 carbon atoms, preferably from 1 to 3;

X_O^I is X as above defined, or equal to X_2^I wherein X_2^I is equal to OH, CH_3, Cl, $N(-CH_2-CH_3)_2$, SCH_2F, SH, or



(s-v)

8. Compounds according to claim 7 wherein R" in the formula (S-I) is $-CO-CH_2OH$, or $-CH(CH_3)-CH_2-CH_2-COOH$.
9. Compounds according to claims 7 and 8 wherein the precursor steroids are those having the hydroxyl function in position 3 and/or in position 11, and/or having in R" an hydroxyl or carboxylic function in terminal position.
10. Compounds according to claims from 7 to 9 wherein the precursor steroids are selected from the following: Budesonide, Hydrocortisone, Alclomethasone, Algestone, Beclomethasone, Betamethasone, Chloroprednisone, Clobetasol, Clobetasone, Clocortolone, Cloprednol, Cortisone, Corticosterone, Deflazacort, Desonide, Desoximethasone, Dexamethasone, Diflorasone Diflucortolone, Difluprednate, Fluazacort, Flucloronide, Flumethasone, Flunisolide,

Fluocinolone Acetonide, Fluocinonide, Fluocortyn Butyl, Fluocortolone, Fluorometholone, Fluperolone Acetate, Fluprednidene Acetate, Fluprednisolone, Flurandrenolide, Formocortal, Halcinonide, Halobetasol Propionate, Halomethasone, Halopredone Acetate, Hydrocortamate, Loteprednol Etabonate, Medrysone, Meprednisone, Methylprednisolone, Momethasone Furoate, Paramethasone, Prednicarbate, Prednisolone, Prednisolone 25-Diethylaminoacetate, Prednisolone Sodium Phosphate, Prednisone, Prednival, Prednylidene, Rimexolone, Triamcinolone, Triamcinolone Acetonide, 21-Acetoxypregnenolone, Cortivazol, Amcinonide, Fluticasone Propionate, Mazipredone, Tixocortol, Triamcinolone Hexacetonide, Ursodesoxycholic acid, Chenodeoxycholic acid, Mitatrienediol, Moxestrol, Ethynylestradiol, Estradiol, Mestranol.

11. Compounds or salts, or their compositions according to claims from 1 to 10 for use as medicaments;
provided that in formula (I) when X_2 of B is a linear or branched C_1 - C_{20} alkylene or a cycloalkylene having from 5 to 7 carbon atoms optionally substituted,
the drugs of formula A = R-T_1 - with the free valence saturated as above described, used in the compound of formula (I), has not to belong to the following classes: drugs for use in incontinence, antithrombotic drugs (ACE inhibitors), prostaglandins, antiinflammatory drugs (NSAIDS and corticosteroids) but not excluding from the antiinflammatory NSAIDS paracetamol and sulindac.

12. Use of the compounds or salts, or their compositions according to claims 1-10 for the preparation of drugs for the therapeutic stress oxidative application;
including, when X_2 of B is a linear or branched C_1 - C_{20} alkylene or a cycloalkylene having from 5 to 7 carbon atoms, the drug of formula A = R-T_1 -, with the free valence saturated as described in claim 1, belonging to the following classes: drugs for use in incontinence, antithrombotic drugs (ACE inhibitors), prostaglandins, antiinflammatory drugs.

13. Pharmaceutical formulations containing as active principle the compounds or their salts according to claims 1-10.

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(54) Title: PROCESS FOR THE PREPARATION OF NAPROXENE NITROXYALKYLESTERS

(57) Abstract: A process for obtaining nitroxyalkylesters of the 2-(S)-(6-methoxy-2-naphthyl)-propanoic acid having an enantiomeric excess higher than or equal to 95 %, preferably higher than or equal to 98 %, characterized in that an halide of the 2-(S)-(6-methoxy-2-naphthyl)-propanoic acid of formula A-Hal, wherein A is the acid acyl residue, is reacted in an inert organic solvent with an aliphatic nitroxyalkanol HO-Y-ONO_2, wherein Y is a C_2-C_{20} alkylene or a cycloalkylene from 3 to 8 carbon atoms, or an alkylene as defined containing a cycloalkylene as defined, in the presence of an inorganic base.

PROCESS FOR THE PREPARATION OF NAPROXENE NITROXYALKYLESTERS

* * * * * *

The present invention relates to a new method for preparing nitroxyalkylesters of the 2-(S)-(6-methoxy-2-naphthyl)-propanoic acid (naproxene) having an enantiomeric excess of the (S) form higher than or equal to 97%, preferably higher than or equal to 98%, combined with high yields, higher than 75-80%, preferably higher than 85%.

It is well known in the prior art that the enantiomeric form (S) is the active form from the pharmacological point of view of the above mentioned product.

In the prior art synthesis methods of nitroxyalkylesters of the 2-(S)-(6-methoxy-2-naphthyl)-propanoic acid, are known. In the patent application WO 98/25,918, a synthesis method of naproxene nitroxyalkyl esters containing in the alkyl chain a saturated C_3-C_8 cycloalkyl residue, is described. In said process the acid or one of its functional derivatives, for example, chloride or anhydride, is reacted, in an inert organic solvent, with a nitroalkanol containing a cycloalkyl residue as above defined. The reaction takes place in the presence of an organic nitrogenated base, such as for example 4-dimethyl aminopyridine, morpholine, N-methyl morpholine or triethylamine. Tests carried out by the Applicant have shown

that this process of the prior art does not allow to obtain naproxene nitroxyalkylesters having an enantiomeric excess in the range of 55-80%, only with a specific organic base, 4-N,N-dimethylamino pyridine, 94% is obtained.

The need was therefore felt to obtain naproxene nitroxyalkylesters having an higher enantiomeric excess, at least of 97%, preferably equal to or higher than 98%.

An object of the present invention is a process to obtain nitroxyalkylesters of the 2-(S)-(6-methoxy-2-naphthyl)-propanoic acid having an enantiomeric excess higher than or equal to 97%, preferably higher than or equal to 98%, characterized in that an halide of the 2-(S)-(6-methoxy-2-naphthyl)-propanoic acid of formula A-Hal, wherein A is the acylic residue of said acid, is reacted in an inert organic solvent with an aliphatic nitroxyalkanol HO-Y-ONO_2, wherein Y has one of the following meanings:

- a linear or optionally branched C_1-C_{20}, preferably C_2-C_5, alkylene;
- a cycloalkylene with ring from 3 to 8 carbon atoms, preferably from 5 to 7 carbon atoms, said cycloalkylene optionally can be substituted with one or two alkylenes as above defined, and/or with one or more alkyl radicals having in the chain a number of carbon atoms as above defined for alkylene;
- an aromatic residue with ring having 5 or 6 carbon atoms,

said aromatic residue optionally can be substituted with one or two alkylenes as above defined, and/or with one or more alkyl radicals having in the chain a number of carbon atoms as above defined for alkylene, or a -COOH group;

- $-(T)_p-(CH(CH_2ONO_2)-CH_2O)_{nf'}-(T)-$,

$-(T)_p-(CH_2-CH(ONO_2)-CH_2O)_{nf'}-(T)-$,

T being alkylene as above dfeined and p an integer equal to zero or one, alkylene having the above mentioned meaning, nf' is an integer from 1 to 6, preferably from 1 to 4; in the presence of an inorganic base, to give the corresponding nitroxyalkylester of the 2-(S)-(6-methoxy-2-naphthyl)-propanoic acid of formula $A-O-Y-ONO_2$, wherein A and Y are as above defined.

Y can also be a combination of two or more of the mentioned group.

The aliphatic nitroxyalcohol amount on molar basis is in the range 1-2, preferably 1.2-1.5, with respect to that of the acid halide.

With inorganic bases hydroxides, oxides, carbonates and bicarbonates, silicates, aluminosilicates of the alkaline and alkaline-earth metals, or hydroxides, oxides, carbonates and bicarbonates of metals belonging to the group IIB, preferably zinc, or to groups IIIa or IVa, preferably tin, are meant.

The inorganic base amount is in molar ratio with the acid

halide amount generally in the range 1-2, preferably 1.2-1.5.

With inert organic solvent according to the present invention aromatic hydrocarbons are meant, such as for example toluene and xylene, chlorinated or fluorinated organic solvents, for example methylene chloride, chlorobenzene, aliphatic esters for example C_1-C_4 acids esters with C_1-C_5 alcohols such as for example ethyl acetate and butyl acetate, etc.

The solvent amount is not critical and generally from 1 to 10 volumes of solvent are used, preferabaly from 2 to 5 volumes based on the acid halide weight.

The reaction is carried out at a temperature in the range -20°C and 50°C, preferably 0°C and 20°C.

The nitroxyalkylesters of the 2-(S)-(6-methoxy-2-naphthyl)-propanoic acid are recovered at the end of the reaction, after addition of water to the organic phase, separation of the phases and solvent evaporation. If necessary, a further purification can be carried out by chromatography on silica gel column in order to increase the product titre.

Alternatively, the compound can also be purified by crystallization from a suitable solvent.

Aliphatic nitroxyalcohols can be prepared according to the known methods in the prior art. See for example Gazzetta Chim. It. 1987, 117, 173 and WO 98/25,918.

The Applicant has found that surprisingly by the use of

inorganic bases it is possible to improve the enantiomeric excess of naproxene nitroxyalkylesters with respect to the prior art methods, which use, as seen, organic bases, with high yields as above mentioned.

The following examples have the purpose to illustrate the invention and they are not to be intended as limitative thereof.

EXAMPLE 1 (comparative)

Preparation of 4-nitroxybutyl ester of the 2-(S)-(6-methoxy-2-naphthyl)-propanoic acid according to WO 98/25918

A mixture of the 2-(S)-(6-methoxy-2-naphthyl)-propanoic acid (0.32 g, 1.4 mmoles), 4-N,N-dimethylamino pyridine (16 mg, 0.13 mmoles), 4-nitroxybutan-1-ol (0.34 g, 2.5 mmoles) in dichloromethane (6 ml) at a temperature in the range 0°C-5°C is added, under stirring, to a solution of N,N'-dicyclohexylcarbodiimide (0.29 g, 1.4 mmoles) in dichloromethane (6 ml). The mixture is left under stirring at the same temperature for 3 hours and then dried by solvent evaporation under vacuum. The residue is purified by chromatography on silica gel column (eluent dichloromethane) to give the 4-nitroxybutyl ester of the 2-(S)-(6-methoxy-2-naphthyl)-propanoic acid (0.41 g, 1.19 mmoles), yield 85%) in the form of an oil. HPLC purity: 98%.

^{1}H NMR($CDCl_3$) δ (ppm): 1.59 (d, 3H, J=7.5 Hz); 1.65 (m, 4H); 3.85 (q, 1H, J=7.5 Hz); 3.91 (m, 2H); 4.10 (m, 2H); 7.1-7.7

(m, aromatic, 8H).

Enantiomeric excess: 94%.

EXAMPLE 2

To a solution of 4-nitroxybutan-1-ol (2.0 g; 14.8 mmoles) in dichloromethane (20 ml), cooled at 0°C-5°C, potassium carbonate (3.21 g, 23.2 mmoles) is added under stirring.

To the mixture a solution of 2-(S)-(6-methoxy-2-naphthyl)-propanoic acid chloride (3.86 g, 15.5 mmoles; enantiomeric excess 98%) in dichloromethane (22 ml) is added, maintaining the temperature in the range 10°C-15°C. When the addition is over the temperature is increased and maintained for 10 hours at a value in the range 15°C-20°C and then the solution is filtered. The solvent is evaporated under vacuum. The residue is purified by chromatography on silica gel column (eluent dichloromethane) to give the 4-nitroxybutyl ester of the 2-(S)-(6-methoxy-2-naphthyl)-propanoic acid (4.4 g, 12.6 mmoles, yield 85%) in the form of an oil. HPLC purity: 99%.

^{1}H NMR($CDCl_3$) δ (ppm): 1.59 (d, 3H, J=7.5 Hz); 1.65 (m, 4H); 3.85 (q, 1H, J=7.5 Hz); 3.91 (m, 2H); 4.10 (m, 2H); 7.1-7.7 (m, aromatic, 8H).

Enantiomeric excess: 98%.

EXAMPLE 3

Example 2 is repeated using toluene as solvent. The nitroxyester yield is 76%, the (HPLC) purity > 99%. The enatiomeric excess is equal to 98%.

EXAMPLE 4

Example 2 is repeated but using as a base calcium carbonate. 4.6 g, equal to 13.3 mmoles of nitroxyester (yield 90%) are obtained, HPLC purity >99%, enantiomeric excess 98%.

EXAMPLE 5

Example 2 is repeated but using as a base calcium alumino-silicate. 4.6 g, equal to 13.3 mmoles of nitroxyester (yield 90%) are obtained, HPLC purity >99%, enantiomeric excess 98%.

EXAMPLE 6

To a solution of 4-nitroxybutan-1-ol (2.0 g; 14.8 mmoles) in dichloromethane (20 ml), cooled at a temperature in the range 0°C-5°C, potassium carbonate (3.21 g, 23.2 mmoles) is added under stirring.

To the mixture a solution of 2-(S)-(6-methoxy-2-naphthyl)-propanoic acid chloride (3.86 g, 15.5 mmoles, enantiomeric excess 98%) in dichloromethane (22 ml) is added, maintaining the temperature in the range 10°C-15°C. When the addition is over, the temperature is increased to a value in the range 15°C-20°C for 10 hours and then the solution is filtered. Water (1 ml) and N,N-dimethylformamide (2 ml) are added to the solution and left under stirring at room temperature for 3 hours. At the end the organic phase is separated, washed with water and filtered through a potassium carbonate panel. The solvent is evaporated under vacuum and 4.1 g, equivalent to 11.8 mmoles of ester (yield 80%) in the form of an oil, are

obtained, HPLC purity >99%, enantiomeric excess 98%.

EXAMPLE 7 (comparative)

Example 2 is repeated but using as a base triethylamine. The obtained mixture after the reaction is analyzed to evaluate the enantiomeric excess, which results equal to 80%.

EXAMPLE 8 (comparative)

Example 2 is repeated but using as a base diisopropylethylamine. The mixture obtained after the reaction is analyzed to evaluate the enantiomeric excess, which results equal to 76%.

EXAMPLE 9 (comparative)

Example 2 is repeated but using as a base N-methylmorpholine. The mixture obtained after the reaction is analyzed to evaluate the enantiomeric excess, which results equal to 56%.

- $-(T)_p-(CH(CH_2ONO_2)-CH_2O)_{nf'}-(T)-$,

$-(T)_p-(CH_2-CH(ONO_2)-CH_2O)_{nf'}-(T)-$,

T being alkylene as above defined and p an integer equal to zero or one, alkylene having the above mentioned meanaing, nf' is an integer from 1 to 6, preferably from 1 to 4;

in the presence of an inorganic base, to give the corresponding nitroxyalkylester of the 2-(S)-(6-methoxy-2-naphthyl)-propanoic acid of formula $A-O-Y-ONO_2$, wherein A and Y are as above defined.

2. A process according to claim 1, wherein the aliphatic nitroxyalcohol amount on molar basis is in the range 1-2, preferably 1.2-1.5, with respect to that of the acid halide.

3. A process according to claims 1 and 2, wherein the inorganic bases are hydroxides, oxides, carbonates and bicarbonates, silicates, aluminosilicates of the alkaline and alkaline-earth metals, or hydroxides, oxides, carbonates and bicarbonates of metals belonging to the group IIB, preferably zinc, or to groups IIIa or IVa, preferably tin.

4. A process according to claims 1-3, wherein the inorganic base amount is in molar ratio with the acid halide amount in the range 1-2, preferably 1.2-1.5.

5. A process according to claims 1-4, wherein the reaction is carried out at a temperature in the range -20°C and 50°C, preferably 0°C and 20°C.

INTERNATIONAL SEARCH REPORT

Int. .onal Application No
PCT/EP 00/07222

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C203/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BEILSTEIN Data, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 30641 A (NICOX LTD) 16 November 1995 (1995-11-16) examples 1C,1H ---	1-5
A	WO 97 16405 A (NICOX SA) 9 May 1997 (1997-05-09) example 3 ---	1-5
A	WO 92 01668 A (ITALFARMACO SPA) 6 February 1992 (1992-02-06) page 5, line 19 - line 29; claim 1 ---	1
A	FR 2 757 159 A (HOECHST MARION ROUSSEL INC) 19 June 1998 (1998-06-19) cited in the application claim 7; example 5 --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 November 2000	24/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bonnevalle, E

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte. onal Application No
PCT/EP 00/07222

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 09831 A (NICOX LTD) 13 April 1995 (1995-04-13) example 1	1

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/EP 00/07222

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9530641 A	16-11-1995	IT 1269735 B	15-04-1997
		IT 1274609 B	18-07-1997
		AT 168986 T	15-08-1998
		AT 184589 T	15-10-1999
		AU 702662 B	25-02-1999
		AU 2215695 A	29-11-1995
		AU 678063 B	15-05-1997
		AU 7809294 A	01-05-1995
		BR 9407749 A	12-02-1997
		BR 9507634 A	23-09-1997
		CA 2173582 A	13-04-1995
		CA 2190087 A	16-11-1995
		DE 69412109 D	03-09-1998
		DE 69412109 T	21-01-1999
		DE 69512232 D	21-10-1999
		DE 69512232 T	24-02-2000
		DK 722434 T	16-11-1998
		DK 759899 T	20-12-1999
		WO 9509831 A	13-04-1995
		EP 0722434 A	24-07-1996
		EP 0759899 A	05-03-1997
		ES 2120070 T	16-10-1998
		ES 2139199 T	01-02-2000
		GR 3032078 T	31-03-2000
		HU 74446 A	30-12-1996
		HU 75961 A	28-05-1997
		JP 9503214 T	31-03-1997
		JP 9512798 T	22-12-1997
		RU 2136653 C	10-09-1999
		SI 722434 T	31-12-1998
		SI 759899 T	31-12-1999
		US 5700947 A	23-12-1997
		US 5861426 A	19-01-1999
		US 5780495 A	14-07-1998
WO 9716405 A	09-05-1997	IT MI952263 A	30-04-1997
		AT 193883 T	15-06-2000
		AU 709338 B	26-08-1999
		AU 7495096 A	22-05-1997
		BR 9611175 A	30-03-1999
		DE 69608916 D	20-07-2000
		EP 0871606 A	21-10-1998
		ES 2148808 T	16-10-2000
		HU 9802986 A	28-04-1999
		JP 11514636 T	14-12-1999
		SI 871606 T	31-08-2000
		US 6040341 A	21-03-2000
WO 9201668 A	06-02-1992	IT 1243367 B	10-06-1994
		AT 118478 T	15-03-1995
		AU 8097491 A	18-02-1992
		CA 2087442 A	27-01-1992
		DE 69107459 D	23-03-1995
		DE 540544 T	23-09-1993
		DK 540544 T	26-06-1995
		EP 0540544 A	12-05-1993
		ES 2056783 T	16-10-1994
		GR 93300079 T	31-08-1993

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. onal Application No
PCT/EP 00/07222

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9201668 A		HU 63374 A	30-08-1993
		HU 213405 B	30-06-1997
		NO 930215 A	22-01-1993
		US 5589490 A	31-12-1996
		US 5366992 A	22-11-1994
FR 2757159 A	19-06-1998	WO 9825918 A	18-06-1998
WO 9509831 A	13-04-1995	GB 2283238 A	03-05-1995
		IT 1269735 B	15-04-1997
		AT 168986 T	15-08-1998
		AU 678063 B	15-05-1997
		AU 7809294 A	01-05-1995
		BR 9407749 A	12-02-1997
		CA 2173582 A	13-04-1995
		DE 69412109 D	03-09-1998
		DE 69412109 T	21-01-1999
		DK 722434 T	16-11-1998
		EP 0722434 A	24-07-1996
		ES 2120070 T	16-10-1998
		HK 1004916 A	11-12-1998
		HU 74446 A	30-12-1996
		JP 9503214 T	31-03-1997
		RU 2136653 C	10-09-1999
		SI 722434 T	31-12-1998
		US 5700947 A	23-12-1997
		US 5780495 A	14-07-1998
		AT 184589 T	15-10-1999
		AU 702662 B	25-02-1999
		AU 2215695 A	29-11-1995
		BR 9507634 A	23-09-1997
		CA 2190087 A	16-11-1995
		DE 69512232 D	21-10-1999
		DE 69512232 T	24-02-2000
		DK 759899 T	20-12-1999
		WO 9530641 A	16-11-1995
		EP 0759899 A	05-03-1997
		ES 2139199 T	01-02-2000
		GR 3032078 T	31-03-2000
		HU 75961 A	28-05-1997
		JP 9512798 T	22-12-1997
		SI 759899 T	31-12-1999
		US 5861426 A	19-01-1999

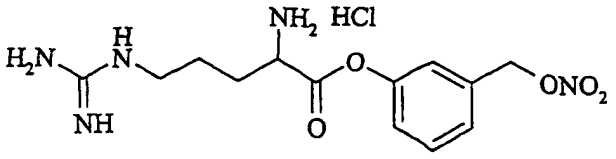
CLAIMS

1. A process for obtaining nitroxyalkylesters of the 2-(S)-(6-methoxy-2-naphthyl)-propanoic acid having an enantiomeric excess higher than or equal to 97%, preferably higher than or equal to 98%, characterized in that an halide of the 2-(S)-(6-methoxy-2-naphthyl)-propanoic acid of formula A-Hal, wherein A is the acyl residue of the acid, is let react in an inert organic solvent with an aliphatic nitroxyalkanol $HO-Y-ONO_2$, wherein Y has one of the following meanings:

- a linear or optionally branched C_1-C_{20}, preferably C_2-C_5, alkylene, or
- a cycloalkylene with ring from 3 to 8 carbon atoms, preferably from 5 to 7 carbon atoms, said cycloalkylene optionally substituted with one or two alkylenes as above defined, and/or with one or more alkyl radicals having in the chain a number of carbon atoms as above defined for alkylene;
- an aromatic residue with ring having 5 or 6 carbon atoms, said aromatic residue optionally substituted with one or two alkylenes as above defined, and/or with one or more alkyl radicals having in the chain a number of carbon atoms as above defined for alkylene, or a -COOH group;

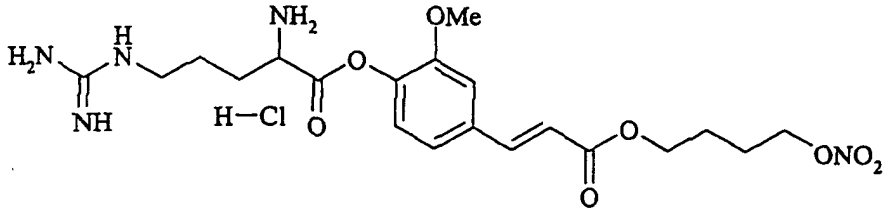
(XXIII)

2-amino-5-guanidinopentanoic acid, 3-(nitrooxy methyl)phenyl hydrochloride ester (XXIV)



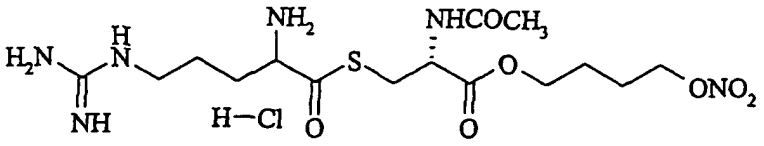
(XXIV)

2-amino-5-guanidinopentanoic acid-, 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxy-1-propenyl]phenyl hydrochloride ester (XXV)



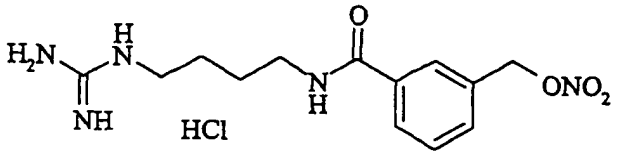
(XXV)

(S)-N-acetylcysteine-4-(nitrooxy)butyl ester, 2-amino-5-guanidinopentanoate hydrochloride (XXVI)



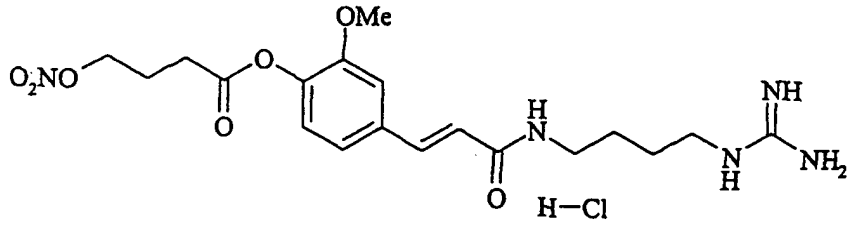
(XXVI)

4-(guanidine)butyl-3-nitrooxymethylbenzamide (XXVII)



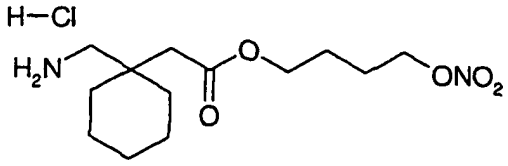
(XXVII)

4-(guanidine)butyl-3-[4-(4'-nitrooxybutyryloxy)-3-(methoxy)]phenyl-2-propenamide chloride (XXVIII)



(XXVIII)

1-(aminomethyl)cyclohexan acetic acid 4-(nitroxy) butyl hydrochloride ester (XXIX)



(XXIX)

12. Compounds according to claims 1-11, as nitrate salts.
13. Compounds according to claims 1-12, in combination with NO-donor compounds.
14. Compounds according to claim 13, wherein the NO donor compounds contain in the molecule radicals of drugs belonging to the classes of aspirin, ibuprofen, paracetamol, naproxen, diclofenac, flurbiprofen.

15. Analgesic drugs for the treatment of the chronic pain, in particular the neuropathic pain, in combination with NO donor compounds.

16. Analgesic drugs according to claim 15, wherein the drug is selected from the following: lamotrigine, topiramate, tiagabime, zonisamide, carbamazepine, felbamate, amineptine, amoxapine, demexiptiline, desipramine, nortriptyline, opipramol, tianeptine, ami-triptyline, butriptyline, clomipramine, dibenzepin, dimetacrine, dothiepin, doxepin, fluacizine, imipramine, iprindole, lofepramine, melitracen, noxiptilin, propi-zepine, protriptyline, trimipramine.

17. Pharmaceutical compositions for parenteral, oral and topical use, comprising the compounds according to claims 1-16.

18. Compounds according to claims 1-17, for use as medicament.

19. Use of the compounds according to claims 1-17, for preparing drugs for the chronic pain, in particular the neuropathic pain.